

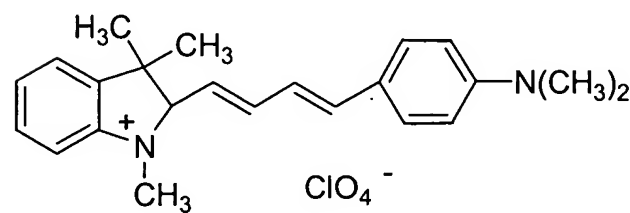
### Listing of Claims:

1. (Currently Amended) A method of staining bacteria comprising: ~~working~~ adding a polymethine dye ~~on~~ to a sample in the presence of a substance capable of reducing nitrite ions to stain bacteria in the sample.

2. (Original) A method according to claim 1, wherein the substance capable of reducing nitrite ions is selected from the group consisting of: ascorbic acid, isoascorbic acid, aminomethanesulfonic acid, aminoethanesulfonic acid, glutamic acid, aspartic acid, mercaptoacetic acid, 3-mercaptopropionic acid, sulfamic acid, sulfanilic acid, sulfurous acid, pyrosulfurous acid, phosphinic acid, glycine, glutamine, asparagine, methionine, glutathione, cysteine, hydroxylamine and salts thereof; sulfanilamide; aminomethane; mercaptoethanol; thiophenol and urea.

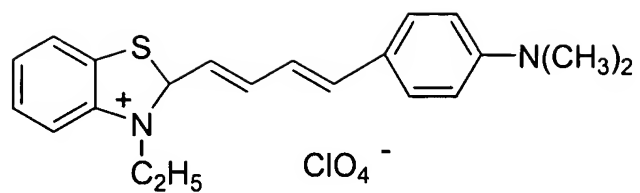
3. (Currently Amended) A method according to claim 1, wherein the polymethine dye is at least one selected from the following group consisting of:

(1) Thiazole Orange;

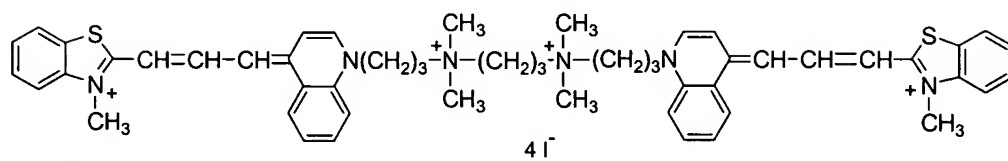
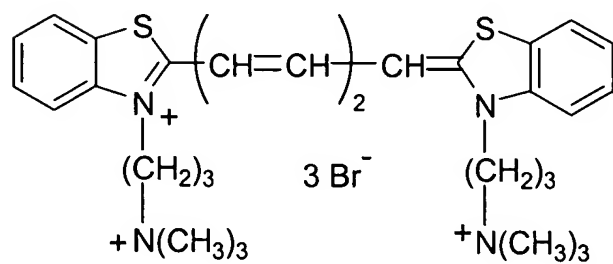


(2)

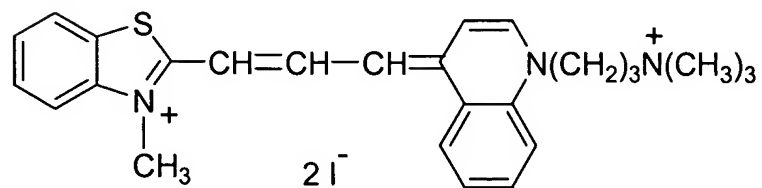
(3)



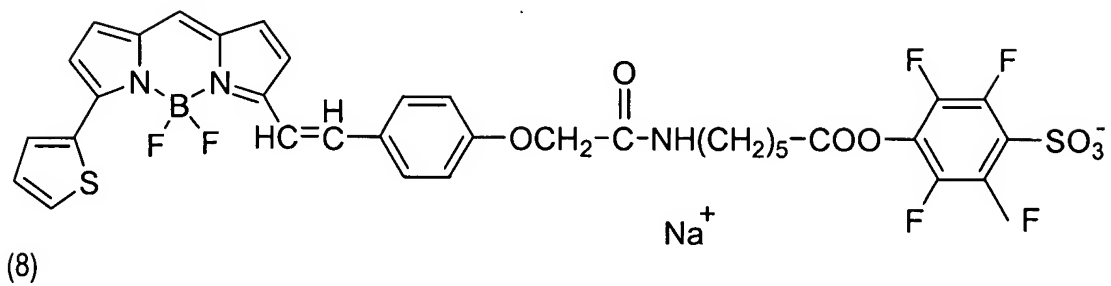
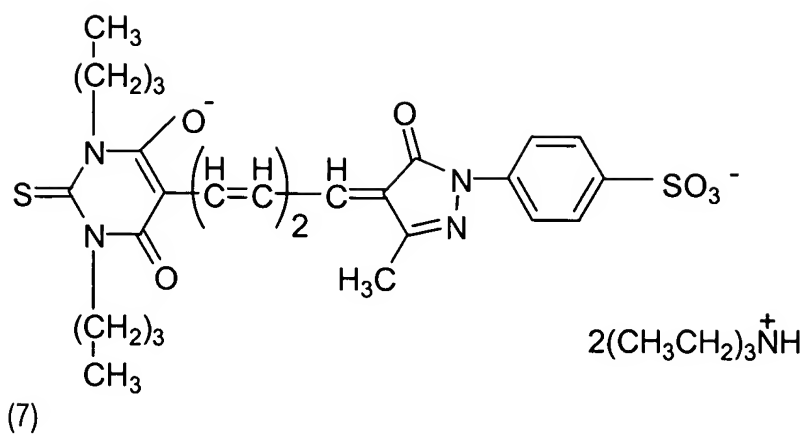
(4)



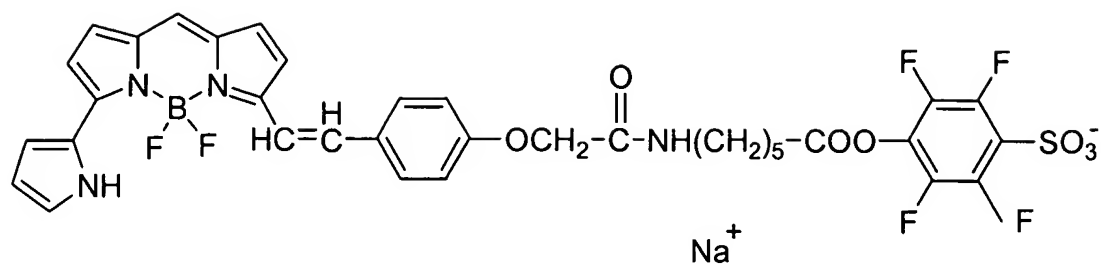
(5)



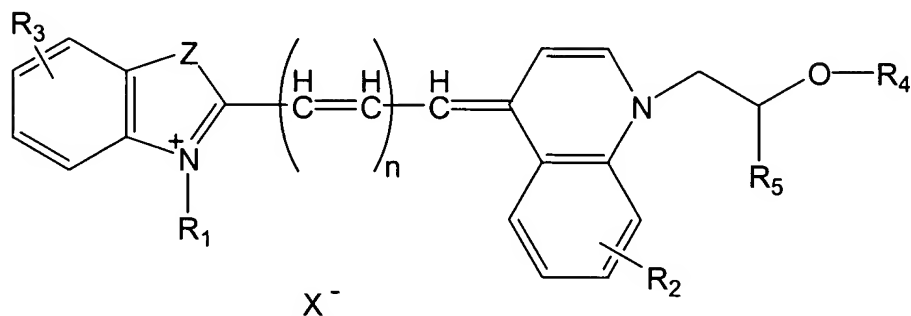
(6)



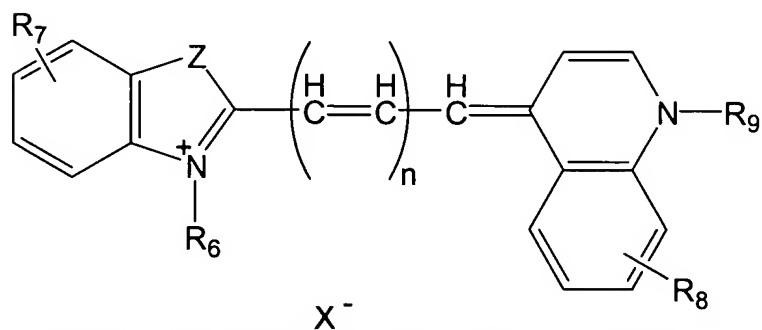
(9)



(10) a compound represented by the following general formula:



wherein  $\text{R}_1$  is a hydrogen atom or a  $\text{C}_{1-3}$  alkyl group;  $\text{R}_2$  and  $\text{R}_3$  are a hydrogen atom, a  $\text{C}_{1-3}$  alkyl group or a  $\text{C}_{1-3}$  alkoxy group;  $\text{R}_4$  is a hydrogen atom, an acyl group or a  $\text{C}_{1-3}$  alkyl group;  $\text{R}_5$  is a hydrogen atom or a  $\text{C}_{1-3}$  alkyl group which may be substituted;  $\text{Z}$  is a sulfur atom, an oxygen atom or a carbon atom substituted with a  $\text{C}_{1-3}$  alkyl group;  $n$  is 1 or 2;  $\text{X}^-$  is an anion; and

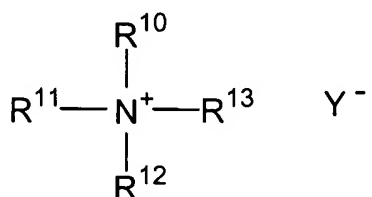


(11) a compound represented by the following general formula:

wherein  $R_4$   $R_6$  is a hydrogen atom or a C<sub>1-18</sub> alkyl group;  $R_2$   $R_7$  and  $R_3$   $R_8$  are a hydrogen atom, a C<sub>1-3</sub> alkyl group or a C<sub>1-3</sub> alkoxy group;  $R_4$   $R_9$  is a hydrogen atom, an acyl group or a C<sub>1-18</sub> alkyl group; Z is sulfur, oxygen or carbon having a C<sub>1-3</sub> alkyl group; n is 0, 1 or 2; ~~X~~ X is an anion.

4. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of a cationic surfactant.

5. (Currently Amended) A method according to claim 4, wherein the cationic surfactant is a quaternary ammonium salt represented by the following formula:



wherein  $R^{10}$  is a C<sub>6-18</sub> alkyl group or (C<sub>6</sub>H<sub>5</sub>)-CH<sub>2</sub>-;  $R^{11}$ ,  $R^{12}$  and  $R^{13}$ , the same or different, are a C<sub>1-3</sub> alkyl group or a benzyl group; ~~Y~~ Y is a halogen ion.

6. (Original) A method according to claim 5, wherein the quaternary ammonium salt is at least one selected from the group consisting of: decyl trimethyl ammonium salt, dodecyl trimethyl ammonium salt, tetradecyl trimethyl ammonium salt, hexadecyl trimethyl ammonium salt and octadecyl trimethyl ammonium salt.

7. (Currently Amended) A method according to claim 1, wherein the ~~dye is worked under~~ sample is in an acidic state.

8. (Original) A method according to claim 7, wherein the acidic state is set at pH 2.0-4.5.

9. (Currently Amended) A method according to claim 4 Z, wherein a buffer of pKa 1-5.5 is used to maintain an acidic pH.

10. (Original) A method according to claim 9, wherein the buffer is at least one selected from the group consisting of: citric acid-NaOH, potassium dihydrogen phosphate-disodium hydrogen phosphate, potassium dihydrogen phosphate-NaOH, citric acid- disodium hydrogen phosphate, potassium hydrogen phthalate-NaOH, succinic acid-NaOH, lactic acid-NaOH,  $\epsilon$ -aminocaproic acid-HCl, fumaric acid-HCl,  $\beta$ -alanine-NaOH and glycine-NaOH.

11. (Currently Amended) A method according to claim 1, ~~wherein the working is carried out in the existence with~~ presence of an inorganic salt of either sulfate or nitrate.

12. (Original) A method according to claim 1, wherein the dye is worked at 0.1 to 100 ppm in the sample.

13. (Currently Amended) A method according to claim 1, wherein the substance capable of reducing nitrite ions ~~exists~~ is present in the sample in such an amount that it can reduces the nitrite ions produced by bacteria of  $10^5$  to  $10^8$ /ml.

14. (Original) A method according to claim 1, wherein the cationic surfactant exists at 10 to 30000 mg/l in the sample.

15. (Original) A method according to claim 10, wherein the acid or the compound maintaining an acidic pH exists at 10 to 500 mM in the sample.

16. (Original) A method according to claim 1, wherein the sample is a urine, blood or spinal fluid.

17. (Currently Amended) A method of detecting and counting bacteria comprising the following steps of:

(1) ~~working~~ adding a polymethine dye ~~on to~~ to a sample by a method as described in ~~any one of claims~~ claim 1 to stain bacteria in the sample,

(2) introducing the ~~thus~~ treated sample into a detecting part of a flow cytometer and irradiating cells of the stained bacteria one by one with light to measure scattered light and fluorescent light emitted from each of the cells; and

(3) discriminating the bacteria from other components in accordance with an intensity of a scattered light signal and an intensity of a fluorescent light signal or a pulse width reflecting the length of particles to count the number of the bacteria.

18. (Original) A method according to claim 17, wherein the step (1) is carried out by the steps of

(a) mixing a sample with an aqueous solution containing a substance capable of reducing nitrite ions and/or a cationic surfactant to accelerate dye transmissivity of bacteria;

(b) staining the bacteria for a predetermined period with a polymethine dye;

19. (Original) A method according to claim 17, wherein the step (3) of discriminating and counting the bacteria is carried out in accordance with at least one selected from the following combinations of:

(i) a forward scattered light intensity and a forward scattered light pulse width;

(ii) a forward scattered light intensity and a fluorescent light intensity; and

(iii) a forward scattered light pulse width and a fluorescent light intensity.